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journal or publication title	The proceedings of the Hoshi University
number	45
page range	139-153
year	2003
URL	http://id.nii.ac.jp/1240/00000112/

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Phosphodiesterases in the vascular system

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Abstract

Cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP) are second messengers involved in the intracellular signal transduction of a wide variety of extracellularstimuli in several tissues. In the vascular system, these nucleotides play important roles in the regulation of vascular tone and in the maintenance of the mature contractile phenotype in smooth muscle cells. At least 11 different gene families of PDEs are currently known to exist in mammalian tissues. Most families contain several distinct genes, and many of these genes are expressed in different tissues as functionally unique alternative splice variants. This article reviews many of the important aspects about the structure, cellular localization, and regulation of each family of PDEs. Particular emphasis is placed on new information obtained in the last few years about vascular diseases. The development of novel methods to deliver more potent and selective PDE inhibitors to individual cell types and subcellular locations will lead to new therapeutic uses for this class of drugs in diseases of the vascular system.

Abbreviations

ACh, acetylcholine; CaM, calmodulin; cAMP, cyclic adenosine 3',5'-monophosphate; cGMP, cyclic guanosine 3',5'-monophosphate; DEAE, diethylaminoethyl; EDHF, endothelium-derived hyperpol-arizing factor; FMD, flow-mediated dilation; GAF, cGMP-regulated cyclic nucleotide PDEs, certain adenylyl cyclases, and the bacterial transcription factor FhlA; GTP, guanine triphosphate; IBMX, 3-isobutyl-1-methylxanthine; NF-kB, nuclear factor-kappaB; NO, nitric oxide; ODQ, 1H-(1,2,4) oxadiazolo[4,3-a]quinozalin-1-one; PAS, PER-ARNT-SIM; PDE, phosphodiesterase; PGI_2, prostacyclin; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; UCR, upstream conserved region; SAH, subarachnoid hemorrhage; SMCs, smooth muscle cells; SNP, sodium nitro-prusside; STZ, streptozotocin

Introduction

The second messengers cyclic nucleotides (cAMP, cGMP) play pivotal regulatory roles in a wide variety of signal transduction pathways and in various tissues (Beavo, 1995). For example, they mediate processes such as vision, olfaction, platelet aggregation, aldosterone synthesis, insulin secretion, T cell activation, and smooth muscle relaxation. In particular, in the vascular system they play important roles in the regulation of vascular tone and in the maintenance of the mature contractile phenotype in smooth muscle cells. The intracellular levels of cAMP and cGMP are tightly controlled both by their rate of synthesis (by adenylyl and guanylyl cyclases, respectively) in response to extracellular signals, and by their rate of [hydrolysis by cyclic nucleotide phosphodiesterases (PDEs). PDEs form a superfamily of enzymes that catalyze the hydrolysis of 3', 5'-cyclic nucleotides to the corresponding nucleotide 5'-monophosphates (which do not activate cyclic nucleotides' effector proteins) (Figure 1).

To date -- on the basis of their substrate specificities, kinetics, allosteric regulators, inhibitor sensitivities, and amino acid sequences -- at least 11 distinct PDE families have been identified, in total containing more than 50 different PDE enzyme variants, each encoded by several genes (Beavo, 1995; Soderling and Beavo, 2000) (Figure 2). Furthermore, each family, and even members within a family, exhibits distinct tissue, cell, and subcellular expression patterns. They are hence likely to participate in discrete signal transduction pathways and thus in discrete physiological and pathophysiological processes, e.g., penile erection, asthma, pulmonary hypertension, atherosclerosis, heart failure, and diabetes. Consequently, PDEs are of both fundamental and pharmacological interest.

Here, we review recent advances relating to the structure, distribution, and regulation of PDEs and their role in vascular cell signaling, and we shall conclude by highlighting possible applications of PDEs to the treatment of vascular disorders.

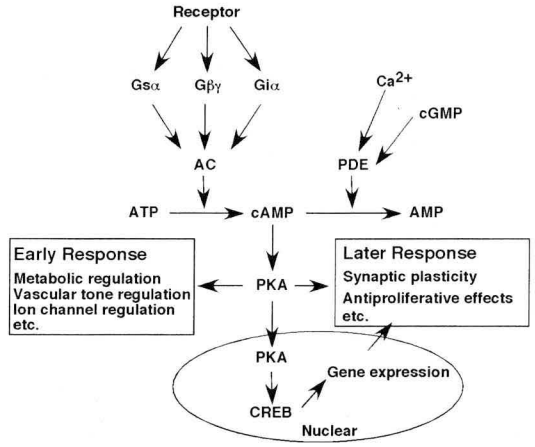

Figure 1. cAMP signaling. Transmembrane receptors of various hormone are coupied to adenylyl cyclase (AC) via heterotrimeric G-proteins. Ligand binding to the receptor changes the receptor conformation, allowing it to associate with a G-protein. This results in the activation of the specific G-protein via exchange of GTP for GDP bound to the α-subunit of the G-protein. The activated G-protein in turn activates AC resulting in the conversion of ATP to cAMP. AC can couple with both the stimulatory and the inhibitory G-proteins (Gs and Gi, respectively). Interaction with Gs stimulates its activity and interaction with Gi inhibits its enzymatic activity. cAMP then acts to regulate a wide variety of cellular processes via activation of protein kinase A (PKA). cAMP is hydrolyzed by phosphodiesterase (PDE). When PKA signaling is transfer to nuclei, various gene expressions are induced via CREB. CREB, the Ca^{2+}/cAMP response element binding protein, is a transcription factor that is a down-stream target of cAMP-PKA signaling.

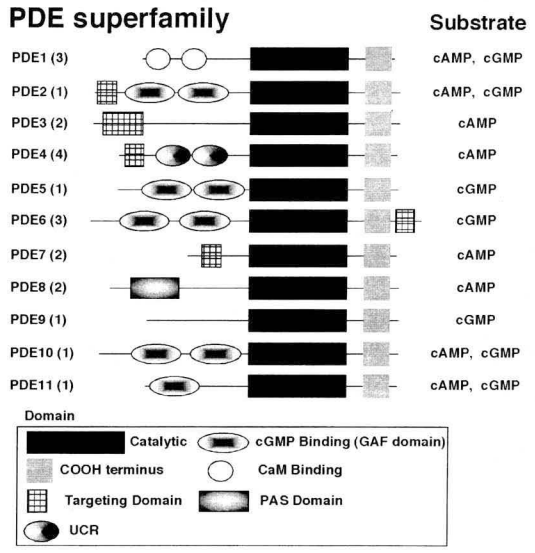

Figure 2. Structural organization of different PDE families. The number in parentheses next to the gene family indicates the number of known genes. In the PDE6 families, only the genes codingfor catalytic subunits are reported. PDE6 can exist as a $\alpha\beta\gamma 2$ heterotrimer of $\alpha\beta$ catalytic and γ inhibitory subunits ; α and β subunits are products of different but closely related genes.

Classification of PDEs

1) PDE1 family

The PDEs in the PDE1 gene family are dependent on calcium-calmodulin (CaM) for activity, and were previously termed CaM-PDEs (Figure 2). Three different PDE1 gene products have been cloned: PDE1A, PDE1B, and PDE1C. The first two, PDE1A and PDE1B, hydrolyze cGMP more efficiently than cAMP, whereas PDE1C hydrolyses cAMP and cGMP with equal efficiency (Beavo, 1995). PDE1A and PDE1B have been extensively characterized (Bentley et al., 1992). Two splice variants of the PDE1A gene, PDE1A1 and PDE1A2, have been isolated from bovine heart and brain, respectively (Sonnenburg et al., 1993, 1995). PDE1A1 and PDE1A2 encode the bovine heart 59-kDa and bovine brain 61-kDa CaM-PDE isozymes, respectively, and they differ only in their N-termini (Novack et al., 1991). For PDE1B1, which encodes the bovine brain 63-kDa CaM-PDE isozyme, only one mRNA product has been isolated so far (Bentley et al., 1992; Repaske et al., 1992). Five PDE1C splice variants, PDE1C1 to 5, have been identified in human and mouse tissues (Loughney et al., 1996; Yan et al., 1996). In the vascular system, PDE1 activity has been demonstrated by DEAE ion exchange chromatography in soluble extracts from a variety of vascular smooth muscle sources, including bovine (Lugnier et al., 1986; Ivorra et al., 1992; Ahn et al., 1992), canine (Pagani et al., 1992; Silver et al., 1988), guinea pig (Silver et al., 1988), human (Lugnier et al., 1986; Hidaka and Endo, 1984), porcine (Saeki and Saito, 1993), rabbit (Hagiwara et al., 1984; Ahn et al., 1989), and rat (Lugnier et al., 1986; Souness et al., 1989) aortas, as well as bovine (Weishaar et al., 1986) and pig (Keravis et al., 1980; Wells et al., 1975) coronary arteries, rat mesenteric arteries (Komas et al., 1991), and human saphenous vein (Pagani et al., 1992).

The "classical" PDE1A isozymes (59- and 61-kDa CaM-PDEs) have been reported to have values for the Michaelis constant (Km) for cAMP within the range 34-40 μM, and Km values for cGMP within the range 2-3 μM (Wang et al., 1990). The maximum velocity (Vmax) ratio for cAMP/cGMP is ~2 for PDE1A isozymes. The PDE1B isozymes (63-kDa CaM-PDEs) have reported Km values for cAMP and cGMP of ~11 and 1 μM, respectively, but a Vmax ratio of only 0.3. In general, a wide range of kinetic constants for CaM-PDE activity has been reported (Wang et al., 1990).

The PDE1 gene products are called Ca^{2+}/CaM-dependent PDEs because they require both Ca^{2+} and

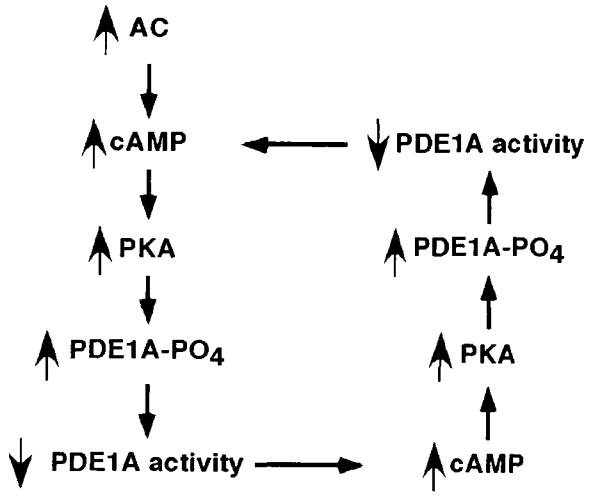

Figure 3. Model for "feedforward" regulation of PDE1A. Model for positive feedforward regulation of cAMP levels by phosphorylation and inhibition of PDE1A by cAMP-dependent protein kinase (PKA). AC, adenylyl cyclase.

CaM for activity. Of interest is the fact that the affinity for Ca^{2+}/CaM is different among the different PDE proteins. CaM antagonists inhibit PDE1 activity, although their lack of PDE selectivity has limited their utility. The PDE1A gene encodes two splicing variants, PDE1A1 and PDE1A2. CaM is 10 times more potent in activating A1 than A2, indicating that splicing is regulating the sensitivity to Ca^{2+} and CaM (Sonnenburg et al., 1995). Additional data comparing isoenzymes from brain, heart, and lung have shown differences in the affinity of PDE1B and PDE1C for CaM (Yan et al., 1996). CaM binding is also regulated by PDE1 phosphorylation. At least three of the CaM-dependent PDEs are regulated in vitro by phosphorylation/dephosphorylation. Both the 59-kDa heart isozyme and the 61-kDa brain isozyme (PDE1A1 and PDE1A2) are phosphorylated by cAMP-dependent protein kinase (Hashimoto et al., 1989; Sharma and Wang, 1985) (Figure 3). The 63-kDa isozyme (PDE1B) is phosphorylated by CaM kinase II (Hashimoto et al., 1989). This phosphorylation is accompanied by a decrease in the isozyme affinity towards CaM-Ca^{2+}, and can be reversed by a CaM-dependent phosphatase (Sharma and Wang, 1985; Hashimoto et al., 1989).

2) PDE2 family

To date, only one gene has been identified for the PDE2 family (Figure 2); however, at least two different 5'-splice variants are known (Yang et al., 1994). Both cAMP and cGMP are hydrolyzed by PDE2, and in fact the Vmax values for both are very similar (Martins et al., 1982). The two substrates show positively cooperative kinetic effects, with a contribution of the two having Hill coefficients of 1.9 and 1.3 for cAMP and cGMP, respectively. PDE2 has not been detected in most of the studies on vascular smooth muscle isozymes, although a very low PDE2 activity has been reported in soluble extracts of porcine aorta (Saeki and Saito, 1993).

3) PDE3 family

PDE3s, purified to apparent homogeneity from a variety of tissues, can be distinguished from other PDEs by their high affinities for both cAMP and cGMP, with Km values within the range 0.1-0.8 μM, and a Vmax for cAMP 4-10 times higher than that for cGMP (Beavo, 1995). So far, two different gene products have been identified as being part of the PDE3 family (Meacci et al., 1992; Taira et al., 1993; Degerman et al., 1997) (Figure 2). The first, PDE3A, has been identified in smooth muscle, platelets, and cardiac tissues. The second, PDE3B, is most abundant in adipocytes and liver. Both forms are found in smaller amounts in other tissues. PDE3s are activated when phosphorylated either by cAMP-dependent protein kinase (PKA) or by phosphatidyl inositol-3-phosphate-dependent protein kinase (Manganiello and Degerman, 1999). In addition, cAMP-elevating agents increase PDE3 activities and levels in several cell types (Degerman et al., 1997), including cultured rat and human aortic vascular smooth muscle cells (Rose et al., 1997; Liu and Maurice, 1998; Palmer and Maurice, 2000). PDE3 isoforms are found in a variety of intracellular locations, being predominantly membrane-associated in adipocytes (Degerman et al., 1997), cytosolic in platelets (Degerman et al., 1994), and cytosolic as well as associated with the sarcoplasmic reticulum in the myocardium (Kauffman et al., 1986; Muller et al., 1992; Smith et al., 1993). Specific PDE3 inhibitors promote smooth muscle relaxation, stimulate myocardial contractility, and inhibit platelet aggregation, suggesting the involvement of PDE3 in the regulation of these physiological (Degerman et al., 1996) and pathophysiological processes (see below).

4) PDE4 family

cAMP-specific PDE (PDE4) activity that is insensitive to cGMP and/or that is inhibited by rolipram and Ro-20-1724 has been demonstrated in a number of vascular smooth muscle tissues. To date, more than

16 different PDE4, cAMP-specific isoforms have been identified (Houslay et al., 1998; Conti et al., 2003). Four separate genes (A, B, C, and D) encode these various isoforms, with additional multiplicity due to alternative mRNA splicing and the use of different promoters (Figure 2). Perhaps because of the weak vasorelaxation caused by PDE4 inhibitors (Polson and Strada, 1996), the expressions of PDE4s in blood vessels have not been studied extensively. In the vascular system, PDE4 activity has been found in bovine (Ivorra et al., 1992; Prigent et al., 1988), porcine (Saeki and Saito, 1993), and rat (Komas et al., 1991) aortas and in rat mesenteric (Komas et al., 1991) and human pulmonary (Rabe et al., 1994) arteries. It was recently reported that two PDE4D "long forms" (PDE4D3, PDE4D5) are expressed in rat and human vascular smooth muscle cells (Liu and Maurice, 1999; Liu et al., 2000). In cultured rat and human aortic vascular smooth muscle cells, incubation with cAMP-elevating agents induces expressions of two PDE4D "short forms": PDE4D1 and PDE4D2 (Liu et al., 2000). In addition to its effect on PDE4D3 activity (Liu and Maurice, 1999; MacKenzie et al., 2000; Baillie et al., 2001), the mitogen-activated protein kinase cascade also regulates *PDE4D* expression, inhibiting cAMP-induced increases in the PDE4D "short forms" through a mechanism involving mRNA destabilization (Liu et al., 2000). Selective and regulated targeting of PDE4s also regulates the impact of these enzymes on cell function (Beard et al., 1999; Liu and Maurice, 1999; McPhee et al., 1999; Yarwood et al., 1999; Grange et al., 2000; Conti et al., 2003). PDE3 and PDE4 activities are elevated after incubation with cAMP-elevating agents, and this increase attenuates the responses to further stimulation with cAMP-elevating agents both in vitro (Rose et al., 1997; Liu and Maurice, 1999; Liu et al., 2000; Palmer and Maurice, 2000) and in vivo (Tilley and Maurice, 2002). These findings need to be discussed in terms of the notion that agents aimed at specific PDE3 or PDE4 variants may allow greater control of the cAMP-mediated regulation of those vascular smooth muscle cells behaviors that are phenotype-dependent.

Recently, it has been reported that mice deficient in PDE4D exhibit delayed growth as well as reduced viability and female fertility. The decrease in fertility of the null female is caused both by impaired ovulation and by diminished sensitivity of the granulosa cells to gonadotropins. Thus, a critical and indispensable role of PDE4 and its regulation in cell homeostasis have been demonstrated (Jin et al., 1999).

5) PDE5 family

A cGMP-binding, cGMP-specific PDE (PDE5) is abundant in lung, platelets, and vascular smooth muscle (Lincoln et al., 1976; Francis et al., 1980; Hamet and Coquil, 1978; Coquil, 1983; Francis, 1985; Hamet and Tremblay, 1988). PDE5 is highly specific for cGMP, both in its catalytic site, and in the two cGMP-binding allosteric sites located within the amino-terminal half of the protein (Thomas et al., 1992; McAllister-Lucas et al., 1995) (Figure 2). In smooth muscle, nitric oxide (NO) and natriuretic peptides regulate vascular tone by inducing relaxation through stimulation of cGMP synthesis (Sausbier et al., 2000). Degradation of cGMP is controlled by PDEs, and PDE5 is the most highly expressed cGMP-hydrolyzing PDE in these cells (Figure 4). The physiological importance of PDE5 in the regulation of smooth muscle tone has been demonstrated most clearly by the clinical use of its specific inhibitor, sildenafil (Viagra®), in the treatment of erectile dysfunction (Ballard et al., 1998). Recently, the development of other drugs targeting PDE5 in vascular smooth muscle has also been reported. For example, both tadalafil (Cialis™) and vardenafil (Levitra™) can specifically inhibit PDE5 activity in the nanomolar concentration range, but they differ somewhat in their inhibitory profiles towards PDEs from other families (Saenz de Tejada et al., 2001; Eardley and Cartledge, 2002). PDE5 is a multi-domain protein that appears to be regulated intricately by phosphorylation as well as by the binding of cGMP to allosteric cGMP-binding sites at the N-terminus of the protein (Turko et al., 1998; Rybalkin et al., 2003). On the basis of sequence homology in the full-length PDE5 protein, it appears that there are two cGMP-binding domains arranged in tandem that (a) follow an N-terminal domain harboring a site for phosphorylation, and (b) precede the C-terminal catalytic domain of the enzyme (McAllister-Lucas et al., 1993). PDE5 is a dimer, and regions mediating the dimerization have been suggested to be present in the allosteric cGMP-binding domain (Martinez et al., 2002; Thomas et al., 1990; Muradov et al., 2003). The cGMP-binding sites of PDE5 have been found to be necessary for the enhancement of Ser92 (bovine PDE5) phosphorylation by PKA or PKG in vitro (Thomas et al., 1990). As a result of PDE5 phosphorylation, the Kd for cGMP binding shifted from 0.13 to 0.03 μM (Corbin et al., 2000). Recently, it was suggested that PDE5, partially purified from rat cerebellum, was allosterically activated by cGMP when a fluorescent analog of cGMP was

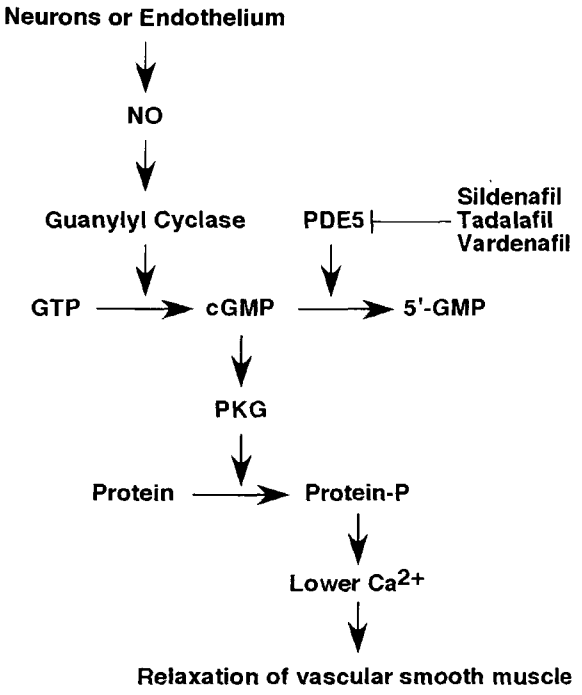

Figure 4. Nitric oxide and cGMP signaling in smooth muscle cells. Tissue cGMP levels are determined by a balance between the activities of the guanylyl cyclases that catalyze formation of cGMP from GTP and the cyclic nucleotides PDEsthat catalyze the breakdown of cGMP. The combination of a stimulator of guanylyl cyclase and a cGMP PDE inhibitor such as sildenafil produces synergistic enhancement of tissue cGMP levels.

employed as a substrate (Okada and Asakawa, 2002).

6) PDE6 family

Photoreceptor cGMP phosphodiesterases (PDE6 family) function as effector proteins in vertebrate visual transduction, which is mediated by the rhodopsin-coupled G protein, transducin (Chabre and Deterre, 1989; Yarfitz and Hurley, 1994, Beavo, 1995). Retinal rod PDE6 is composed of two catalytic PDE6 α β subunits, each tightly associated with the smaller inhibitory g subunit (P γ) (Baehr et al., 1979; Hurley and Stryer, 1982; Deterre et al., 1988) (Figure 2). Cone PDE6 consists of two identical PDE α ' subunits complexed with two copies of the cone-specific P γ subunit (Gillespie and Beavo, 1988; Li et al., 1990; Hamilton and Hurley, 1990). The catalytic subunits of rod and cone PDE, as well as the respective P γ subu nits, share a high degree of homology (Hamilton and Hurley, 1990; Lipkin et al., 1990). The key role of Pg is to inhibit cGMP hydrolysis by the catalytic subunits in the dark. Upon light stimulation of photoreceptors, PDE6 is activated by GTP-bound transducin- α , which displaces P γ from the enzyme catalytic core. PDE5 and PDE6 share a common domain organization, i.e., two noncatalytic cGMP-binding sites are located N-terminally to the conserved PDE catalytic domain (McAllister-Lucas et al., 1993). Furthermore, PDE5 and PDE6 display a high homology (45-48% identity) between catalytic domains, a strong substrate preference for cGMP, and similar patterns of inhibition by competitive inhibitors such as zaprinast, dipyridamole, and sildenafil (McAllister-Lucas et al., 1993; Gillespie and Beavo, 1989; Turko et al., 1999; Ballard et al., 1998).

7) PDE7 family

PDE7 was first isolated at the gene level in 1993 from a human glioblastoma cDNA library, and expressed in a cAMP-deficient strain of the yeast *Saccharomyces cerevisiae* (Michaeli et al., 1993). PDE7A encodes a cAMP-specific PDE that is insensitive both to cGMP and to inhibitors of PDE3 and PDE4, and it has an amino acid sequence distinct from those of the other cAMP PDEs (Michaeli et al., 1993) (Figure 2). In humans (Gardner et al., 2000; Hetman et al., 2000; Sasaki et al., 2000) and mice (Gardner et al., 2000; Hetman et al., 2000), two genes (PDE7A and PDE7B) have been identified that encode PDE7. With respect to PDE7A, three isoenzymes (PDE7A1, PDE7A2, and PDE7A3) can theoretically be derived from the same gene by alternative mRNA splicing. PDE7A2 is generated from a 5'-splice variant and, therefore, differs from PDE7A1 in its NH_2-terminal domain (Bloom and Beavo, 1996; Han et al., 1997). In mice and humans, PDE7A2 mRNA is expressed abundantly in skeletal muscle, heart, and kidney, whereas the testis, lung, and immune system (thymus, spleen, lymph node, blood leukocytes) are rich sources of HSPDE7A1, where HS refers to Homo sapiens (Bloom and Beavo, 1996; Han et al., 1997; Wang et al., 2000). In the vascular system, the mRNAs for PDE7A1 and also PDE7A2 and also PDE7A1 protein, have been found to be expressed in vascular smooth muscle cells obtained from lung-derived pulmonary artery (Smith et al., 2003), and the mRNAs for PDE7A1 and PDE7A2 are expressed in vascular endothelial cells (Miro et al., 2000).

8) PDE8 family

PDE8A expression is highest in testis, followed by eye, liver, kidney, skeletal muscle, embryo, ovary, and brain in mice (Soderling et al., 1998). In humans, it has a similar tissue distribution (Fisher et al., 1998a).

PDE8A is specific for the hydrolysis of cAMP, with a low Km of approximately 70 nM (Soderling et al., 1998; Fisher et al., 1998a). PDE8 was the first example (PDE9 is now the second) of a PDE that is not inhibited effectively by 3-isobutyl-1-methylxanthine (IBMX), a non-selective PDE inhibitor. Thus, it should be emphasized that the lack of an effect of IBMX may not necessarily be a useful indicator that PDEs do not regulate a particular physiological function (Soderling and Beavo, 2000). On the basis of sequence homology with a domain found in proteins from bacteria to eukariots, a PAS (Period, Arnt, Sim) domain has been identified in PDE8 (Soderling et al., 1998) (Figure 2). This domain functions as a signal detector, and is usually associated with a heme or a chromophore cofactor (Zhulin et al., 1997). Although the function of the PAS domain in PDE8 is not known, it may be important for protein-protein interaction or for sensing the concentration of a small ligand (Soderling and Beavo, 2000), suggesting a mode of regulation novel among PDEs. To date, it is not known whether PDE8 expression and activities are present within the vascular system.

9) PDE9 family

PDE9 is specific for the high-affinity hydrolysis of cGMP, with a Km of 70 nM (Fisher et al., 1998b; Soderling et al. 1998) (Figure 2). Like PDE8, PDE9 is not effectively inhibited by IBMX. PDE9 is expressed in smooth muscle in the small intestine, as well as in kidney, liver, lung, brain, testis, skeletal muscle, heart, thymus, and spleen (Fisher et al., 1998b; Soderling et al. 1998; Soderling and Beavo, 2000). PDE9A mRNA is widely distributed throughout the brain, with a varying regional expression, and its expression pattern closely resembles that of soluble guanylyl cyclase in the rat brain, suggesting a possible functional association or coupling of these two enzymes in the regulation of cGMP levels (Andreeva et al., 2001). To date, four 5' alternative splice variants have been identified for PDE9; however, the functional implications of these variants remain as yet currently unknown (Guipponi et al., 1998).

10) PDE10 family

The PDE10 family was originally isolated from both human (Fujishige et al., 1999a; Loughney et al., 1999) and mouse (Soderling et al., 1999). These studies demonstrated that PDE10 can hydrolyze both cAMP and cGMP, but may function as a cAMP-inhibited cGMP PDE. PDE10A contains two GAF (cGMP binding and stimulated phosphodiesterases, *Anabaena* adenylate cyclases, and *Escherichia* coli FlhA) domains in the N-terminal (Aravind and Ponting, 1997) and a catalytic domain in the C-terminal portions of the molecule (Fujishige et al., 1999a; Soderling et al., 1999) (Figure 2). The amino-acid sequence of the catalytic domain bears a closer similarity (40-47% identical) to those of human PDE2A (Rosman et al., 1997), PDE5A (Yanaka et al., 1998), PDE6A (Pittler et al., 1990), PDE6B (Collins et al., 1992), PDE6C (Feshchenko et al., 1996), and PDE11A (Yuasa et al., 2000) than to those of other PDEs. These PDEs contain two GAF domains and show rather low sequence similarity (19-32% identities within catalytic domains) to those of other PDE families lacking these domains. Thus, PDE2, PDE5, PDE6s, PDE10A, and PDE11A constitute a group of PDEs containing GAF domains. PDE10A transcripts are particularly abundant in human putamen, caudate nucleus, and testis. In situ hybridization analysis has demonstrated expressions of PDE10A transcripts in striatal neurons in the rat brain (Fugishike et al., 1999a). The presence of PDE10A activities as high-affinity cAMP PDE and cAMP-inhibited cGMP PDE in extracts of rat striatum and testis indicates that PDE10A functions to control cyclic nucleotide levels.

11) PDE11 family

At the moment, the final PDE family is represented by PDE11A, which catalyzes the hydrolysis of both cAMP and cGMP (Fawcett et al., 2000; Yuasa et al, 2000). It has unique splice variants (Yuasa et al, 2000), and a unique structural feature of PDE11A is the presence of multiple forms of GAF (see above) (Figure 2). The amino-acid sequence of the PDE11A catalytic domain situated in the C-terminal moiety bears a closer resemblance (42-51% identical) to those of human GAF-PDEs than to those of other PDEs lacking the GAF domain (27-35% identities). PDE11A4 contains two complete GAF domains, whereas PDE11A3 has one complete and one incomplete GAF domain (Yuasa et al., 2000). On the other hand, PDE11A1 has an incomplete GAF domain that lacks the N-terminal part of the GAF consensus sequence (Fawcett et al., 2000). With regard to tissue-specific expression, PDE11A3 transcripts are specifically expressed in testis, whereas PDE11A4 transcripts are particularly abundant in prostate, suggesting some distinct physiological roles of PDE11A via cyclic nucleotide metabolism in these tissues (Yuasa et al., 2000). To date, it is not known whether PDE11A expression and activities are

present within the vascular system.

Phosphodiesterases in vascular disease

Atherosclerosis

A normal artery consists of quiescent arterial smooth muscle cells (SMCs) covered by a monolayer of endothelial cells lining the interior of the blood vessel. If the artery is injured by an excess amount of atherogenic lipid, by oxidative stress, diabetes (see below), smoking, viruses, or by mechanical means, the SMCs respond by proliferating and forming a neointimal lesion (Ross, 1999). Atherosclerotic lesions occur in the context of endothelial cell dysfunction and involve activation, migration, and proliferation of SMCs. Therefore, considerable effort has been devoted to the identification of factors that regulate SMC proliferation. Endothelial-derived relaxing factors, such as NO or prostacyclin (PGI_2), relax blood vessels and inhibit the proliferation and migration of SMCs by increasing the synthesis of the cyclic nucleotides cAMP or cGMP. In fact, cAMP and cGMP inhibit the proliferation of arterial SMCs (Koyama et al., 2001), and elevation of cyclic nucleotides reduces neointimal formation after angioplasty in animal models. Oral administration for 3-21 days of milrinone (0.3-3.0 mg/kg), a bipyridine derivative that specifically inhibits PDE3, suppressed intimal thickening by up to 56% in a dose- and time-dependent manner in a mouse model of photochemically-induced vascular injury (Kondo et al., 1999). In this model, oral administration of milrinone decreased the number of activated SMC and consequently suppressed intimal thickening by preventing SMC proliferation within the media. PDE1C is expressed in proliferating human SMCs, but is absent from the quiescent human aorta. Inhibition of PDE1C in SMCs isolated from normal aorta or from atherosclerotic lesions, using antisense oligonucleotides or a PDE1 inhibitor, results in suppression of SMC proliferation. Because PDE1C is absent from quiescent SMCs, PDE1C inhibitors may target proliferating SMCs in atherosclerotic lesions or during restenosis (Rybalkin et al., 2002).

Diabetes

Atherosclerosis and other cardiovascular diseases are much more prevalent in diabetics than in the human population at large, and they represent a significant cause of morbidity and early mortality in diabetes (Stern, 1995; Taegtmeyer, 1996; Sowers, 1997). It has been reported that alterations in PDEs occur in diabetes-associated cardiovascular disease (Nagaoka et al., 1998; Netherton et al., 2002). For example, Nagaoka et al (1998) reported an increased PDE3 activity in the aorta of atherosclerosis-prone insulin-resistant cp/cp rats that correlated positively with increase in the amount of PDE3A mRNA. Netherton et al (2002) reported that in this same animal model, arterial SMCs from homozygous obese (cp/cp) rats, but not from age-matched healthy (+/+ or +/cp, collectively termed +/?) littermates, display an "activated" phenotype both in vitro and in vivo, and have an elevated level of PDE activity. Thus, these data are consistent with an increased role for PDE3 in regulating cAMP-dependent signaling in cp/cp SMCs, and they identify PDE3 as having a cellular activity potentially responsible for the phenotype of cp/cp SMCs.

We recently reported that the impaired EDHF-type relaxation in the mesenteric artery that is seen in streptozotocin (STZ)-induced diabetic rats might be attributable to a reduced action of cAMP, in turn resulting from increased PDE3 activity (Matsumoto et al., 2003) (Figure 5). We believe that our findings should stimulate further interest in PDE3 as a potential therapeutic target in the continuing efforts to reduce diabetes-associated vascular disease. In clinical studies, flow-mediated dilation (FMD), induced by occlusion of the brachial artery, is an index of NO-dependent endothelial function, and this is impaired in patients with type 2 diabetes. Desouza et al (2002) assessed the acute and prolonged effects of a low dose of sildenafil (25 mg), an inhibitor of PDE5, on FMD in patients with type 2 diabetes. One hour after oral administration of sildenafil 25 mg, FMD had increased the brachial artery diameter significantly, whereas it did not change with placebo. After treatment with sildenafil 25 mg daily for 2 weeks, and testing 24 hours after the last dose, the mean FMD was found to be 14%. In contrast, the mean FMD with placebo was 9%. These results suggest that acute or prolonged sildenafil treatment has a favorable effect on brachial artery FMD that persists for at least 24 hours after the last dose (Desouza et al., 2002). Further investigation is needed to determine whether this prolonged effect has clinical implications in patients with type 2 diabetes.

Pulmonary hypertension

The pulmonary vascular bed is a low-pressure system with a resistance approximately one-tenth that of the systemic circulation. In the normal lung, pulmonary vascular tone is regulated by a balance between the effects of vasodilators/anti-proliferative

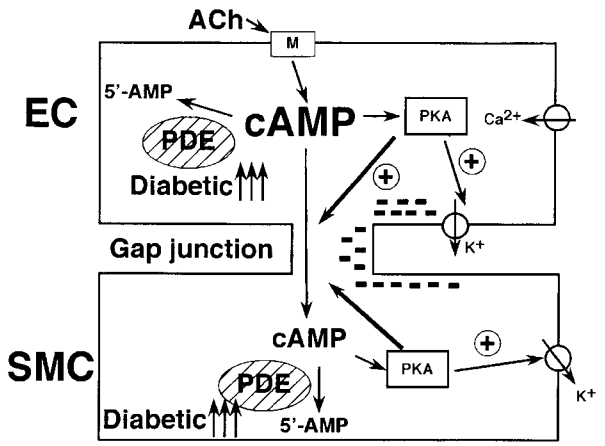

Figure 5. Schematic showing the possible sites of action of cAMP in the mediation of EDHF-type relaxations under physiological and diabetic state. Efflux of K^+ ions results in membrane hyperpolarization. which promotes an increase in cytosolic free Ca^{2+} in the endothelial cell (EC), but a decrease in the smooth muscle cell (SMC). The hyperpolarization of the EC, following Kcachannel activation, can be transmitted along the monolayer of EC or towards the SMC through gap junctions. PDE, phosphodiesterase ; PKA, protein kinase A

agents (such as isoprenaline and PGI_2) and vasoconstrictors/co-mitogens (such as serotonin and endothelin-1). Acute hypoxia causes pulmonary arterial vasoconstriction and increased pulmonary arterial pressure. Chronic hypoxia induces sustained increases in both pulmonary arterial pressure and pulmonary vascular SMC proliferation, and the chronically hypoxic rat is widely used as a model for the study of chronic hypoxia-induced pulmonary hypertension (Jeffery and Wanstall, 2001; Strange et al., 2002). There are several reports suggesting that PDEs play important roles in the development and maintenance of pulmonary hypertension. PDEs activity is increased in pulmonary arteries from rats with chronic hypoxia-induced pulmonary hypertension (MacLean et al., 1997), and this is correlated with decrease in the intracellular cAMP and cGMP levels (MacLean et al., 1996). In chronic hypoxic-treated pulmonary hypertension-model rats, PDE3A/B gene transcript levels have been found to be increased in the main, first, intrapulmonary and resistance pulmonary arteries. PDE5A2 mRNA transcript and protein levels of in the main and first branch pulmonary arteries were also found to be increased by chronic hypoxia, with no effect on PDE5A1/A2 in the intra-pulmonary and resistance vessels. In the same model, it has been suggested that PDE5 expression might be regulated by the NF-κB signaling pathway (Murray et al., 2002). A recent study showed that inhibition of PDE3 and PDE4 activities can significantly improve pulmonary hypertension, and that PDE3 mRNA expression was significantly increased in pulmonary artery rings obtained from Sprague-Dawley rats suffering from hypoxia-induced pulmonary hypertension, while PDE4B mRNA expression tended to be reduced, although not significantly (Wagner et al., 1997). Chronic PDE5 inhibition has been shown to elevate pulmonary cGMP levels and abrogate hypoxia-induced pulmonary hypertension and vascular remodeling in animal models, and to reduce pulmonary artery pressure in primary pulmonary hypertension (Hanasato et al., 1999; Wilkens et al., 2001; Zhao et al., 2001; Michelakis et al., 2002).

Inhaled vasodilators have been shown to achieve selective pulmonary vasorelaxation and supraselective vasodilation in well-ventilated (i.e., inhaled vasodilator-accessible) regions within the lung in both experimental and clinical studies. Inhalation of gaseous NO (Troncy et al., 1998; Papazian et al., 1999) and nebulization of PGI_2 (Walmrath et al., 1993; Olschewski et al., 1996; Walmrath et al., 1996; Zwissler et al., 1996) have been noted both to improve ventilation-perfusion matching and to lower pulmonary arterial pressure in patients suffering from acute respiratory distress syndrome or chronic pulmonary hypertension. The prostanoid PGI_2 doses, however, possess a very short biological half-life (2-3 min) at a physiological pH, and after inhalation of aerosolized PGI_2 the pulmonary vasodilator effect is lost within <30 min both under experimental conditions and when tested in patients (Olschewski et al., 1996; Schermuly et al., 1999). In intact rabbits with acute pulmonary hypertension (Schermuly et al., 1999), subthreshold intravenous doses of nonselective PDE3, PDE4, and PDE5 inhibitors have been noted to augment and prolong the pulmonary vasodilator response to inhaled PGI_2 while limiting the hypotensive effect in the pulmonary circulation. Recently, a prior administration of subthreshold doses of the clinically approved PDE inhibitors theophylline, dipyridamole, and pentoxifylline via the intravascular or inhalational route, which did not itself influence pulmonary hemodynamics, caused more than a doubling of the immediate drop in pulmonary arterial pressure that occurred in response to PGI_2, and also a marked prolongation of the post-PGI_2 vasorelaxation to >60 min (all the PDE inhibitors being effective when given route) (Schermuly et al., 2001). Thus, coaerosolization of PGI_2 and PDE inhibitors should be considered as a

postulated therapeutic strategy against pulmonary hypertension.

Subarachnoid hemorrhage

It is well established that endothelium-dependent, NO-induced cerebral vasodilator responses are impaired in a variety of animal models of subarachnoid hemorrhage (SAH) (Kanamaru et al., 1989; Kim et al., 1989; Edwards et al., 1992; Sobey et al., 1996), and also in patients with SAH (Hatake et al., 1992; Onoue et al., 1995). This may be partly due to reduced NO release resulting from damaged to endothelial cells (Smith et al., 1985; Clower et al., 1994), but importantly it seems that other mechanisms may also contribute. For example, numerous studies have reported that the cerebral vasodilator responses to NO-donor drugs are also impaired after SAH (Kim et al., 1989; Onoue et al., 1995; Zuccarello et al., 1996; Yamamoto et al., 1997), suggesting that the responsiveness of cerebral vascular smooth muscle to NO is altered. Moreover, the vasodilator responses to ACh, sodium nitroprusside (SNP), and low concentrations of zaprinast, an inhibitor of PDE5, are all impaired in SAH rats (Sobey and Quan, 1999). In contrast, the vasodilator responses to adenosine and 8Br-cGMP were similar between control and SAH rats, and the vasoconstrictor response to ODQ, an inhibitor of soluble guanylate cyclase, were unaffected by SAH. In the presence of zaprinast, the responses to ACh and SNP were similar between control and SAH rats. In a canine model of SAH, PDE5 activity was increased to above control levels within the basilar artery seven days after SAH, and the PDE5 expression was most prominent in SMCs following SAH (Inoha et al., 2002). Thus, an increased rate of cGMP hydrolysis by PDE5 may be a major factor contributing to the impairment of NO-mediated cerebral vasodilation after SAH. On the other hand, in a canine model of acute cerebral vasospasm, BRL61063, rolipram, and denbufylline, a selective inhibitor of PDE4, reversed the basilar artery spasm produced by autologous blood without altering mean arterial blood pressure. In contrast, prolonged treatment with BRL61063 failed to alter the development of basilar spasm in two canine hemorrhage models of chronic cerebral vasospasm. Denbufylline-induced relaxation in vitro was also significantly impaired in basilar arteries obtained from a model of chronic vasospasm. In conclusion, PDE4 appears to be the predominant isozyme regulating vascular tone via cAMP hydrolysis in cerebral vessels (Willette et al., 1997). Further investigation is needed to determine whether inhibition of PDEs activity might be a useful approach in patients with SAH.

In conclusion, we believe that manipulation of the activities of PDEs within vascular system may have considerable therapeutic potential. Once the full repertoire of the PDEs expressed within the vascular system has been established, it should not be long before new generations of selective PDE inhibitors are available to manipulate vascular cell responses.

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